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RESEARCH ARTICLE

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Effects of salicylic acid (SA), ultraviolet radiation (UV-B and UV-C) on trans-resveratrol inducement in the skin of harvested grape berries

Abstract Effects of salicylic acid (SA), ultraviolet radiation (UV-B and UV-C) on the trans-resveratrol (Res) inducement of the skin of harvested grape berries were studied with three grape cultivars Takasuma, Tano Red and Carigane. Split plot design tests were adopted to compare the effects of UV-B and UV-C radiation on Res inducement of different cultivars. Results showed that spraying 100 mg·L^{-1} SA markedly enhanced Res contents in the skins of harvested berries for the three selected cultivars. However, the effect of SA varied with the cultivars, and Res inducement by SA was more effective to Tano Red than Takasuma and Carigane. UV-B or UV-C irradiation significantly increased Res contents in grape skins and UV-C was more effective than UV-B. The effects of UV types and dosages on Res inducement depended upon cultivars. In the range of 0–3.6 kJ·m^{-2}, the Res contents in the skins of the three grape cultivars were enhanced along with the increase of dosages of UV-B and UV-C.

Keywords grape, salicylic acid (SA), ultraviolet radiation, UV-B, UV-C, trans-resveratrol

1 Introduction

Trans-Resveratrol (Res, 3,4′,5-trihydroxystilbene) is one of the important phenolic compounds. It was found in *Vitis* L. in 1976 and considered as a grapevine phytoalexin in response to epiphyte infection, mechanical damage and UV irradiation (Langcake and Pryce, 1976). Epidemiological studies showed that Res had a significant effect in cancer chemoprevention and in reducing the risk of coronary heart disease (Jang et al., 1997; Fremont, 2000). The research and application of Res in grape have been paid more attention to in scientific researches and corporation production because grape and grape products have relatively higher Res contents, with abundant grape germplasm resources, and the Res extracts from grapes possess pure natural characteristics, which can meet the requirement of curatorial and sanitarian products.

Secondary metabolite contents such as Res will be increased sharply when grape suffers from an abiotic intimidation (Zhao et al., 2005). Ultraviolet radiation is considered to be an abiotic intimidation. It can be divided into three parts according to wavelength: UV-A (315–390 nm), UV-B (280–315 nm) and UV-C (shorter than 280 nm). UV-C irradiation can significantly enhance the Res contents in grape berries (Langcake and Pryce, 1977; Celine et al., 1999; Li et al., 2004; Rong et al., 2005). Currently, the effect of UV-B irradiation on the Res inducement is unclear. Also, secondary metabolite synthesis such as the Res synthesis in the grapevine can be regulated by signal molecules (Zhao et al., 2005). Salicylic acid (SA) is a low molecular weight phenolic compound and a kind of important signal molecule in response to adversity in plants (Liu et al., 2005). Very few research reports about the effects of SA on the Res inducement in grape berry skins have been published.

The aim of this study is to analyze the effects of SA on the Res inducement as well as the discrepancy of effects of UV-B and UV-C on the Res inducement in the skin of harvested grape berries, which can provide a theoretical support for man-made control of Res synthesis.

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2 Materials and methods

2.1 Plant materials

Ripe berries of three grape cultivars Takasuma, Carigane and Tano Red used in this study were collected from the Grape Germplasm Repository in the Institute of Botany of the Chinese Academy of Sciences in 2004. All cultivars were planted in the spring of 1993. The vines, trained to bilateral cordons, had 1.5 m plant spacing and 2.5 m row spacing in a north-south row orientation, with better growth and fruiting. The vineyard was moderately managed.

Sampling of Takasuma, Carigane and Tano Red was conducted on August 30, September 25 and September 10 in 2004, respectively. Berries without damnification, diseases and insect pests, and in uniform ripeness, color and size were selected to have SA spraying or UV irradiation treatment immediately. One replication per treatment for Takasuma, Carigane and Tano Red required 15, 15 and 30 berries, respectively.

2.2 SA and UV treatment

2.2.1 SA inducement

100 mg·L^{-1} of SA was sprayed on the surface of the chosen berries and the grape berries treated with the double-distilled water were taken as control. All treatments were repeated 3 times. The berries were laid for 24 h in the dark at $(20 \pm 2)^{\circ}$C in the growth chamber after the berries were air-dried in the dark. The berries were peeled by hand, and frozen in liquid nitrogen, ground to powder, and then stored at -40°C.

2.2.2 UV irradiation

Grape berries were placed on lacker plates on the incubating shelf installed with three UV-B or UV-C lamps. We acquired 200 μW·cm^{-2} of UV-B and 400 μW·cm^{-2} of UV-C by regulating the height of sample plates. Three different dosages of UV-B or UV-C (1.2 kJ·m^{-2}, 2.4 kJ·m^{-2}, and 3.6 kJ·m^{-2}) were acquired by illuminating for a different length of time (UV-B 10 min, UV-C 5 min; UV-B 20 min, UV-C 10 min; UV-B 30 min, UV-C 15 min), respectively. Grape berries were overturned once every 2.5 min to get uniform UV-B or UV-C irradiation. The grape berries without illumination were taken as control ones. A split plot design was used in this study with cultivar as the main factor (factor A) placed in the main plot, and radiation dosage as subfactor (factor B) placed in the auxiliary plot. Main and auxiliary plots were chosen randomly with 3 replicates. After treatments, the berries were stored for 24 h in the dark at $(20 \pm 2)^{\circ}$C in a growth chamber. The skin of the berries was separated and frozen in liquid nitrogen, ground to powder, and then stored at -40°C.

2.3 Extraction and determination of trans-resveratrol

Trans-resveratrol was extracted and determined according to the method of Li et al. (2006). Three grams of frozen samples of grape skin were precisely weighed and ground using a porcelain mortar and pestle in 15 mL ethyl acetate. The samples were extracted in the dark at 25°C for 24 h and then centrifuged at 10000 × g for 15 min. The supernatant was evaporated to dryness by rotary vacuum evaporation at 45°C. The dried samples were then dissolved in 1 mL methanol, and filtered through a 0.45 μm PTFE membrane filter before HPLC analysis. Res contents were analyzed using the Dionex Summit HPLC system including a Dionex P680 pump, a Dionex TCC-100 thermostated column compartment, and a Dionex PDA-100 detector (Dionex Corp., Sunnyval, CA). Res was separated on an Inertsil ODS-3 column (250 mm L × 4.6 mm I.D., 5 μm particle size, purchased from GL Sciences Inc., Tokyo, Japan) and a guard column cartridge (Great Eur-asia Science & Development Co. Ltd., Beijing, China), and maintained at 25°C. Ten μL sample solution was eluted with 40% acetonitrile (gradient grade, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) at a flow rate of 0.6 mL·min^{-1}. Res was monitored at 306 nm wavelength.

3 Results

3.1 Effects of SA on Res inducement in the skin of harvested grape berries

Figure 1 shows that spraying 100 mg·L^{-1} SA could markedly increase the Res content in the grape skins of

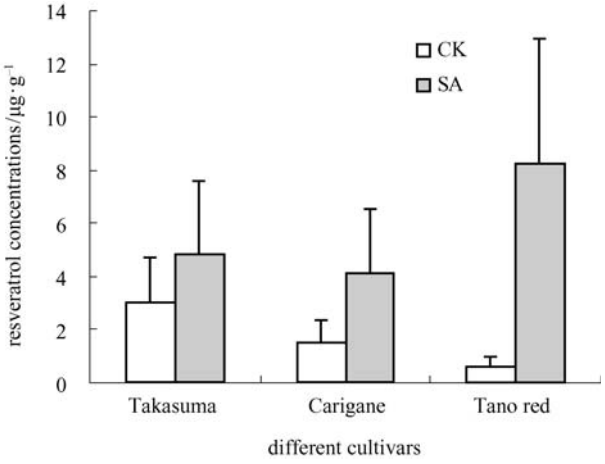

Fig. 1 Resveratrol content in the berry skin of different grape cultivars 24 h after spraying 100 mg·L^{-1} SA
Note: Vertical line on the column of the histogram indicates standard error of different treatments.

the three cultivars, while the Res synthesis in the three cultivars was different. The Res inducement of Tano red with the lowest Res content in the grape berry skin at ripeness was much better than that of the other two cultivars. The Res content of Tano red was 8.21 $\mu g \cdot g^{-1}$, which was 13.6 times of the control samples after spraying SA for 24 h. The Res content in control samples of Takasuma and Carigane was higher than that of Tano red. The Res content in grape berries of Takasuma and Carigane was also significantly increased after SA spraying for 24 h, which was 1.62 and 2.77 times of the control respectively.

3.2 Effects of UV-B and UV-C on Res inducement in the skin of harvested grape berries

Res contents in grape berry skins of the three cultivars were increased by UV-B or UV-C irradiation, but there were differences among different grape cultivars, ultraviolet types and dosages of irradiation. Tests of between-subject effects showed that there were significant differences in the Res contents of different cultivars (factor A) and different irradiation dosages of UV-B or UV-C (factor B). No significant difference was found among three replicates of the same cultivar and same irradiation dosage [C (A)]. The interaction of cultivars and irradiation dosages of UV-B or UV-C (factor A*B) had marked effects on the Res contents in grape berry skins (Table 1).

Table 2 shows that there was significant correlation between the Res inducement and accumulation in harvested grape berry skin by UV-B or UV-C irradiation and grape cultivars. The Res content in Tano Red skins was significantly higher than that in the skins of Takasuma and Carigane after UV-B irradiation for 24 h. However, the Res content in Takasuma skins was significantly higher than that in the skins of Carigane and Tano Red after UV-C irradiation for 24 h, and the Res content in Carigane was higher than that in Tano Red. Furthermore, the effects of UV-C on the Res inducement and accumulation were significantly higher than those of UV-B in the berry skins of the three cultivars; the former was 8.66, 4.41 and 1.31 times of the latter, respectively.

Table 3 shows the response of the Res contents in the berry skins of the three cultivars to different dosages of UV-C or UV-B irradiation. There were no significant differences in the Res contents in Takasuma skins between the selected different dosages of UV-B treatments and the control. Dosages of 2.4 $kJ \cdot m^{-2}$ and 3.6 $kJ \cdot m^{-2}$ of UV-C irradiation could significantly increase the Res contents in Takasuma skins, reaching 39.34 $\mu g \cdot g^{-1}$ and 32.34 $\mu g \cdot g^{-1}$, respectively, which were 13.20 and 10.8 times of the Res contents in the control. As for Carigane, treated with a dosage of 3.6 $kJ \cdot m^{-2}$ of UV-B irradiation, the Res content in the berry skin was the highest (4.56 $\mu g \cdot g^{-1}$), which was 3.06 times of that in the control. However, there were no significant differences in the Res contents between the dosages of 1.2 $kJ \cdot m^{-2}$ and 2.4 $kJ \cdot m^{-2}$ of UV-B irradiation treated grapes and the control. The Res content was increased with the increase of UV-C irradiation dosages in the range of 1.2–3.6 $kJ \cdot m^{-2}$. There were significant differences in the Res contents among different dosages of UV-C irradiation, and the Res contents in grape berry skins treated with all dosages of UV-C irradiation were significantly higher than those of the control. The Res contents in Tano Red were increased with the increase of

Table 1 Tests of between subjects effects of split plot design test of grape cultivars and dosages of UV

variance sources		UV-B			UV-C		
		mean square	*F*	*P*	mean square	*F*	*P*
cultivar (A)	hypothesis	50.685	110.14	0.000	370.027	69.541	0.000
	error	0.460			5.321		
UV dosage (B)	hypothesis	27.745	41.21	0.000	1061.602	295.43	0.000
	error	0.673			3.593		
replicate C (A)	hypothesis	0.460	0.68	0.665	5.321	1.481	0.240
	error	0.673			3.593		
interaction A*B	hypothesis	13.692	20.34	0.000	191.645	53.333	0.000
	error	0.673			3.593		

Table 2 Trans-resveratrol concentrations in the berry skin of different grape cultivars 24 h after UV-B and UV-C irradiation

cultivars	Res contents/$\mu g \cdot g^{-1}$	
	UV-B	UV-C
Takasuma	2.89 B	25.02 A***
Carigane	3.42 B	15.08 B***
Tano Red	8.44 A	11.09 C***

Note: Means followed by the different capital letters are significantly different at $P < 0.01$ level between cultivars under the same UV radiation. Means followed by *** indicate significant different at $P < 0.001$ level between UV-B and UV-C radiation for the same cultivar.

Table 3 Effect of UV dosages on trans-resveratrol concentrations in the berry skins of different grape cultivars

cultivars	UV type	trans-resveratrol concentrations in the grape skin under different dosage of UV radiation/$\mu g \cdot g^{-1}$			
		0 $kJ \cdot m^{-2}$	1.2 $kJ \cdot m^{-2}$	2.4 $kJ \cdot m^{-2}$	3.6 $kJ \cdot m^{-2}$
Takasuma	UV-B	2.98 ab	2.65 b	2.80 ab	3.21 a
	UV-C	2.98 b B	3.37 b B	39.34 a A	32.34 a A
Carigane	UV-B	1.50 b B	3.26 ab AB	2.43 b AB	4.56 a A
	UV-C	1.50 D	8.57 C	14.65 B	22.03 A
Tano Red	UV-B	0.60 c C	7.24 b B	8.14 b AB	9.95 a A
	UV-C	0.60 D	7.51 C	11.10 B	15.87 A

Note: Means followed by the different capital or small letters are significantly different at $P < 0.01$ and $P < 0.05$ levels respectively between different radiation intensities under the same UV radiation for the same cultivar.

the dosages of UV-B and UV-C irradiation in the range of 1.2–3.6 $kJ \cdot m^{-2}$, and were significantly higher than those of the control. The Res contents in grape berry skins, when treated with the dosage of 3.6 $kJ \cdot m^{-2}$ UV-B and UV-C irradiation, were 9.95 $\mu g \cdot g^{-1}$ and 15.87 $\mu g \cdot g^{-1}$, which were 16.58 and 26.45 times of those of the control.

4 Discussion

This study showed that UV-B and UV-C irradiation could induce the Res synthesis in grape berry skins (Table 3). However, the effects of UV-C on the Res inducement in the skins of harvested grape berries was much higher than the effects of UV-B (Table 2), which indicated that ultraviolet wavelength was an important factor in the Res inducement. A series of involved enzymes, such as phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H), *p*-coumaroyl-CoA ligase (4CL) and stilbene synthase (STS) were needed in the Res synthesis through the acetate-malonate and phenylpropanoid pathways (Langcake and Pryce, 1977). STS was the key enzyme in the Res synthesis pathway (Melchior and Kindl, 1991). Fritzemeier and Kindl (1981) reported that the STS activity increased more than one hundred-fold 15 h after UV-C irradiation to the leaves of *Cissus antarctia*r; two other enzymes (PAL, C4H) functioning on the pathway of Res synthesis were induced concurrently, indicating that more Res inducement by UV-C irradiation was acquired through improving the activities of involved enzymes in Res synthesis. Langcake and Pryce (1977) reported that sensitive wavelength of light to STS was 260–270 nm, which was a UV-C spectrum area. This should be the main reason for a less effect of UV-B on the Res inducement than that of UV-C. However, further studies are needed to determine whether there are differences between mechanisms of UV-B and UV-C on the Res inducement in grape berry skins. Moreover, ultraviolet absorbability by the ozonosphere was different according to different light wavelength; absorption coefficients of light wavelength of 360 nm, 320 nm and 280 nm are 1×10^{-3}, 1 and 1×10^{2}, respectively. All of UV-A could go through the ozonosphere, most of UV-B could go to the earth's surface, and almost all of UV-C with a short wavelength and a high energy could be absorbed by the ozonosphere, which means that there is no UV-C irradiation in the Res inducement in grape berry skins. It is necessary to adopt artificial UV-C irradiation on harvested grape berry skins to obtain table grapes and grape products with high Res contents.

Exogenous SA has many physiological functions in plants such as anti-disease inducement. This study showed that SA spraying could induce the Res synthesis in grape skins, with the same effects as damage, epiphyte infection and ultraviolet irradiation (Fig. 1). Though it has been reported that the exogenous SA can improve the PAL activity in cucumber leaves (Shi et al., 2004), not enough evidences exist to interpret the mechanism of exogenous SA inducing the Res synthesis and accumulation in grape berry skins. SA is an important signal molecule responding to the adversity in plants (Liu et al., 2005). Other reports have proved that the endogenous SA level can be increased when plants are under UV irradiation (Yalpani et al., 1994). Therefore, it is an important research problem in future studies to determine whether there is an SA intervening signal transduction in the process of UV inducing the Res synthesis in grape berry skins.

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